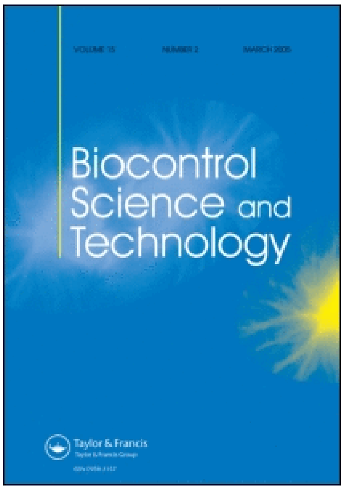

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Justin L. Hatting [a]; Stephen P. Wraight [b];Ray M. Miller [c]
[a] Agricultural Research Council - Small Grain Institute, Bethlehem, South Africa [b] Plant Protection Research Unit, USDA-ARS, Ithaca, NY, USA [c] Pietermaritzburg, University of Kwa Zulu-Natal, Scottsville, South Africa

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Efficacy of *Beauveria bassiana* (Hyphomycetes) for Control of Russian Wheat Aphid (Homoptera: Aphididae) on Resistant Wheat Under Field Conditions

JUSTIN L. HATTING[1], STEPHEN P. WRAIGHT[2] AND RAY M. MILLER[3]

[1]*Agricultural Research Council – Small Grain Institute, P/Bag X29, Bethlehem 9700, South Africa;* [2]*USDA-ARS, Plant Protection Research Unit, Tower Road, Ithaca, NY, USA;* [3]*University of Kwa Zulu-Natal, Pietermaritzburg, P/Bag X01, Scottsville, South Africa*

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The Russian wheat aphid, Diuraphis noxia *(Kurdjumov) (Homoptera: Aphididae) is considered the most important pest of wheat produced under dryland field conditions in South Africa. As part of an integrated pest management strategy, the entomopathogenic hyphomycete* Beauveria bassiana *(Balsamo) Vuillemin was evaluated in combination with antibiotic host plant resistance under dryland field conditions during 1998 and 1999. A commercial formulation, Mycotrol® ES, was applied at a rate of 2.4 L (5×10^{13} conidia) per hectare + 0.1% organosilicone surfactant. During both years, two applications were administered, i.e., on growth stages 31 (first node detectable) and 39 (early flag leaf). An additional treatment, application at growth stage 39 only, was included during 1999. Over the duration of the 1998 trial, ca. 65% fewer aphids were observed on treated plots compared with controls. A similar level of population reduction was observed during the 1999 trial; however, treatment effects were only briefly evident due to a rapid field-wide decline in aphid populations caused by adverse (cool, wet) weather conditions. The early application (GS 31) resulted in some level of control only during 1998. It was hypothesized that this phenomenon was the result of greater exposure to the spray applications and/or greater secondary pick-up of fungal inoculum by the aphids due to the higher level of aphid activity observed on the cultivar employed during that year. In this regard, migration of* D. noxia *onto the flag leaves should be further investigated as a behavioural trait for possible exploitation when considering the use of a mycoinsecticide.*

Keywords: Beauveria bassiana*, control,* Diuraphis noxia*, efficacy, field, Russian wheat aphid*

Correspondence to: J. L. Hatting, ARC-Small Grain Institute, P/Bag X29, Bethlehem, South Africa. Tel.: +27-58-3073437; Fax: +27-58-3073519; E-mail: justin@kgs1.agric.za

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INTRODUCTION

Host-plant resistance against the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), was reported for the first time in South Africa by Du Toit (1987). Numerous resistant wheat cultivars have since been bred for commercial use against this aphid, worldwide (Souza, 1998; Bonjean & Angus, 2001), with 15 such cultivars released in South Africa since 1992 (Tolmay, 2001). In South Africa, these resistant cultivars form the basis of an integrated-control programme being developed by the Small Grain Institute (SGI) of the South African Agricultural Research Council (ARC). By lowering the frequency of chemical control applications, the widespread deployment of host-plant resistance is expected to promote the additive effects of both indigenous and introduced natural antagonists of this aphid (Marasas *et al.,* 1997; Marasas, 1999).

Continued development and integration of biocontrol agents is particularly important in view of the fact that available resistant cultivars are highly variable in their capacity to retain yield potential under severe *D. noxia* infestations (Tolmay, 2002) and also because the potential exists for development of resistance-breaking aphid biotypes. This threat was recently realised in the USA where a new *D. noxia* biotype (biotype B) has appeared that is capable of damaging previously resistant wheat varieties (Moellenberg, 2003).

Aphid control in years when high *D. noxia* pressure occurs may be economically justifiable on cultivars with the lower levels of resistance. However, chemical intervention could jeopardise the long-term objectives of the integrated strategy being developed by ARC-SGI for cereal aphid management. In addition to *D. noxia*, there are at least five other aphid species that damage wheat in South Africa (Annecke & Moran, 1982). More selective pesticides, which would pose low risk to the natural enemy complexes being established to control these pests, are urgently needed.

The potential integration of a biorational insecticide within this programme is underscored by the findings of Knudsen *et al*. (1994). These authors studied the tritrophic interaction between *D. noxia*, the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin, and a non-preferred host plant (oats, *Avena sativa* L.) versus a preferred host plant (wheat, *Triticum aestivum* L.). This interaction was found to be significant for total nymph production, in that the proportionate reduction of the aphid population by the fungus was greater on the non-preferred host. These results support a hypothesis that the efficacy of fungal entomopathogens might be positively affected by the use of resistant wheat varieties. In this regard, reduced leaf rolling (Tolmay *et al.,* 1999) may result in greater exposure of aphids to spray applications; greater tolerance could provide slow-acting fungi with more time to infect and kill the host before economical injury is caused to the crop, while antibiotic resistance will lead to slower development (i.e., extended periods between ecdysis) of the insect pest (Smith, 1989), thereby providing fungi with more time to penetrate the cuticle. Only one other published report of *B. bassiana* efficacy against *D. noxia* on resistant wheat exists. Vandenberg *et al.* (2001) reported that *B. bassiana* strain GHA was ineffective against *D. noxia* infesting the resistant spring wheat cultivar 'IDO-488'.

Field trials were conducted under dryland conditions during the 1998 and 1999 seasons to assess the aphid-control efficacy of spray applications of *B. bassiana* in combination with antibiotic host-plant resistance.

MATERIAL AND METHODS

Trial Design and Layout

Both trials were conducted at the Small Grain Institute of the Agricultural Research Council of South Africa, Bethlehem, Free State Province. During both years, a plot of 800 m^2 (20 $\times$ 40 m) was planted at a seeding density of 25 kg/ha. A different *D. noxia*-resistant cultivar was used in each season. During 1998, cultivar 'Limpopo' (released by ARC-SGI during 1995; Koekemoer & Warburton, 1996) was included; whereas, in 1999, the more recently released cultivar 'Elands' (Koekemoer, 1999) was used. Both wheat cultivars carried the same aphid-resistance gene DN1 (Vicki Tolmay, ARC-SGI, pers. comm.) derived from the antibiotic donor line PI 137739 (Du Toit, 1989a,b). Natural aphid infestations occurred in both years. The two trials were designed as completely randomised designs (Cochran & Cox, 1957) with eight (one treatment and an untreated control, each replicated four times) and 12 (two treatments and an untreated control, each replicated four times) sub-plots, respectively, during 1998 and 1999. Each sub-plot measured 10 m^2 (2.5×4 m).

Fungus and Delivery Rate

A commercial preparation of *B. bassiana* strain GHA formulated as a paraffinic oil-based emulsifiable suspension (Mycotrol® ES produced by Mycotech Corp., Butte, MT, USA; currently Emerald BioAgriculture Corporation) containing 2.1×10^{13} conidia/L was applied at a rate of 2.4 L (5×10^{13} conidia)/ha. Percentage germination, verified prior to application during both years, exceeded 97%. Mycotrol® ES was applied by means of a CO_2-pressurized back-pack sprayer fitted with five single flat-fan nozzles (no. 8001. TeeJet Spray Systems Company, Wheaton, IL, USA) spaced ca. 50 cm apart on a single boom carried at a walking speed delivering 350 L/ha at 200 kPa. Nozzles were directed directly downward, delivering the spray suspension from a height of ca. 15–20 cm above the crop canopy. The organosilicone nonionic surfactant, Break-Thru® (polyether-poly-methylsiloxane-copolymer) (Goldschmidt Chemical Corporation, Hopewell, VA, USA), was added to the spray suspension at a rate of 0.1%, i.e., the registered full-rate for use with certain herbicides under field conditions in South Africa (Syngenta, Reg. no. L5895). Control plots were left untreated.

Spray Applications, Aphid Counts and Data Analyses

During 1998, aphid counts were conducted on five dates, i.e., 10/01 (pre-spray), 10/14, 10/23, 10/30 and 11/06. Mycotrol® ES was applied on growth stage 31 (i.e., first node detectable; 10/02) and again on growth stage 39 (i.e., flag leaf ligule just visible; 10/15) (growth stages according to Tottman, 1987). Both spray applications were administered just after sunset. During the 1999 trial, five counts were again executed, i.e., on 10/04 (pre-spray), 10/18, 10/29, 11/08, and 11/17. Treatment 1 received a Mycotrol® ES application on growth stage 31 (after sunset, 10/05) and again on growth stage 39 (early morning, 10/20) while Treatment 2 received only one spray application on growth stage 39 (early morning, 10/20). During each count, two plants were selected (nondestructively) in each of five rows totalling 10 plants per plot. Selection of plants was performed systematically within each row. On the initial sample date, plants number 5 and 25 were selected in each row. For each subsequent sample, the position was shifted by five plants so that no plant was sampled more than once.

During each count, the following parameters were quantified: (1) number of tillers per plant, (2) number of *D. noxia*-infested tillers per plant, and (3) total number of adult (winged and wingless) and nymphal aphids per tiller. The total number of aphids per 10 plants for each plot was used in the analysis. Data were analysed using the statistical program GenStat for Windows (2000). One way analysis of variance (ANOVA) was used to test for differences between the control and treated plots. However, due to pre-spray differences detected in the total number of *D. noxia* in treatment versus control plots during 1998, an additional analytical method was used to assess potential treatment effects during that year. Usually, the preferred method of analysis of treatment effects under these circumstances would be analysis of covariance (ANCOVA). ANCOVA adjusts the post-spray counts (Y) for the linear relationship with the pre-spray counts (X). An important assumption for the use of ANCOVA, however, is that the regression of aphid density on sample date for each group (treatment versus control) must have a common slope (Snedecor & Cochran, 1980), and in this study there were no significant linear relationships between pre- and post-spray aphid counts ($P > 0.10$). An alternative when ANCOVA is not applicable, is the use of $Y - X$, the change in the count, as the measure of treatment effect (Snedecor & Cochran, 1980). Unfortunately, analysis of $Y-X$ may be inferior to ANCOVA, if the correlation between X and Y is low (Snedecor & Cochran, 1980), and this was also the case in this study. After considering these various difficulties, it was decided to assess potential treatment effects by application of more than one analytical method. Thus, analysis of variance was used to compare changes in pre- versus post-treatment means ($Y-X$, where Y_i = first count post application; Y_{ii} = second count post application; etc.) and also to directly compare unadjusted treatment means per survey date. Moreover, in view of these pre-treatment differences, corrected percentage efficacies were calculated according to the following modification of Abbott's formula as described by Henderson and Tilton (1955):

$$\% \text{ Efficacy} = [1 - (T_a/C_a \times C_b/T_b)] \times 100$$

where T_b is infestation in treated plot prior to application; T_a is infestation in treated plot after application; C_b is infestation in control plot prior to application; C_a is infestation in control plot after application.

Multiple treatment means were separated using the Tukey Honestly Significant Difference (HSD) test at the 5% level of significance (Snedecor & Cochran, 1980). For percentage infested tillers, the arcsine transformation was used, and for number of aphids, the log transformation was used to stabilise treatment variances. For yield comparison (g/plot; Mettler PE 2000 electronic scale) all plots were harvested by hand using conventional sickles. Data were analysed using GenStat for Windows (2000).

Weather Data

Daily weather data were recorded at the Bethlehem Loch Lomond weather station situated at ARC-SGI. During both 1998 and 1999, the wheat fields used in the trials were within 100 m of this station. All weather data were supplied by the Agromet Section of the ARC-Institute for Soil, Climate and Water, Pretoria, South Africa.

RESULTS

1998 Trial

Compared with the pre-spray counts for 1999, the natural aphid infestation within plots varied considerably. Most importantly, the pre-spray count (98/10/01) indicated a significant difference in the total number of *D. noxia* in treatment versus control plots ($P = 0.011$; Table 1).

TABLE 1. Mean number of *D. noxia* recorded per 10 plants pre- and post-application of Mycotrol® ES at a rate of 2.4 liters (5×10^{13} *B. bassiana* conidia) per hectare +0.1% Break-Thru® 1998

Date	98/10/01[a]	98/10/14	98/10/23	98/10/30	98/11/06
Control	32.0 ± 4.1b[b]	113.8 ± 61.3a	120.8 ± 24.5b	49.5 ± 21.8a	16.8 ± 3.4a
Treatment	53.8 ± 12.6a	80.8 ± 18.6a	50.5 ± 20.2a	28.5 ± 11.4a	8.5 ± 7.1a
Efficacy[c]	–	29.0	58.2	42.4	49.4
Corrected efficacy[d]	–	55.5 ± 16.7	74.9 ± 7.9	64.0 ± 17.6	66.5 ± 30.0
P	0.011	0.424	0.005	0.104	0.059
F (1,6 df)	13.26	0.74	19.34	3.67	5.39

[a]Pre-spray count. Spray applications administered on 98/10/02 and 98/10/15.
[b]Log 10 transformation applied prior to ANOVA. Means ± standard deviations followed by the same letter within a column are not significantly different by ANOVA at the 5% level.
[c]Percent reduction in aphid population relative to the control.
[d]Corrected efficacy ± standard deviation calculated according to the formula of Henderson & Tilton (1955).

The comparative change ($Y_i - X$) in the number of *D. noxia*, recorded 12 days post application (i.e., on 98/10/14), was not significantly different in treated versus control plots ($F = 4.64$ on 1,6 df; $P = 0.075$). However, some level of control was evident as the aphid population had increased ca. 3.5-fold on controls compared to a 1.5-fold increase in treated plots. This effect was further amplified following the second application on 98/10/15. During the 8 days following this treatment, the aphid population in the treated plots declined by 38%, while the population in the control plots increased by 6% (Figure 1). These fluctuations ($Y_{ii} - X$) differed significantly ($F = 49.29$ on 1,6 df; $P < 0.001$), as did the actual number of aphids recorded per plot on 10/23 ($P = 0.005$; Table 1).

Expressed as a percentage of the control, efficacy of the treatments averaged 44.8% over the duration of the trial, but this value is likely to be a conservative estimate because of the higher pre-spray aphid densities recorded in the treatments (Table 1). After applying the Henderson–Tilton correction, efficacy averaged ca. 65%. A significant treatment effect was also observed by comparing the cumulative number of aphids recorded over the last three sample dates, i.e., 87.5 versus 187.0 aphids per 10 plants for treatment and control plots, respectively ($F = 15.41$ on 1,6 df; $P = 0.008$).

In contrast to the findings with population densities, no significant difference was detected between the pre-spray infestation levels in the control versus treatment plots ($P = 0.339$; Table 2). No significant differences in percentage infested tillers were detected until day 28, when ca. 50% fewer tillers were found infested compared with controls ($P < 0.001$; Table 2, Figure 2). A 35% lower rate of infestation was recorded in the treatment plots relative to the controls on 10/23. This difference was not significant at the 5% level but was significant at the 10% level ($P = 0.069$), and a highly significant 48% lower rate of infestation was observed in the treatment plots in the subsequent sample (10/30) (Table 2). By day 35 post application, the wheat was less palatable (ca. growth stage 60), resulting in very low aphid densities (< 3 aphids per plant). Subsequently, sampling was terminated following the fifth count on 98/11/06.

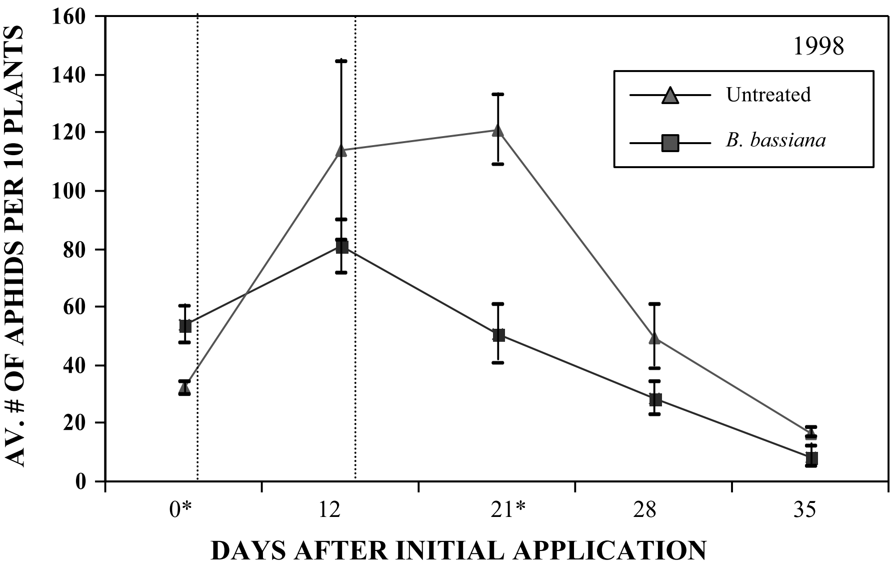

FIGURE 1. Mean number of aphids recorded per 10 plants for trial in 1998. Bars represent Standard Deviation of the Mean. Asterisks indicate significant difference between treatments and controls at the 5% level of significance. Dotted lines indicate Mycotrol® ES applications, i.e., on 98/10/02 and 98/10/15.

TABLE 2. Percentage tiller infestation recorded per 10 plants pre- and post-application of Mycotrol® ES at a rate of 2.4 liters (5×10^{13} *B. bassiana* conidia) per hectare +0.1% Break-Thru® 1998

Date	98/10/01[a]	98/10/14	98/10/23	98/10/30	98/11/06
Control	21.4±3.2a[b]	35.8±7.4a	26.4±6.2a	16.0±2.4b	8.4±3.0a
Treatment	18.9±3.8a	27.3±6.7a	17.1±6.0a	8.3±0.9a	5.5±2.0a
Efficacy[c]	–	23.7	35.2	48.1	34.5
P	0.339	0.135	0.069	< 0.001	0.135
F (1,6 df)	1.08	2.97	4.90	42.99	2.97

[a]Pre-spray count. Spray applications administered on 98/10/02 and 98/10/15.
[b]Arcsine transformation applied prior to ANOVA. Means ± standard deviations followed by the same letter within the same column are not significantly different by ANOVA at the 5% level.
[c]Percentage reduction in the rate of tiller infestation relative to the control.

Weather conditions remained stable throughout the duration of the trial with average daily minimum and maximum temperatures of 10.2°C (range 4.3–14.5°C) and 23.9°C (range 13.9–30.3°C), respectively, measured for the (combined) months of October and November. A single heavy rainfall of 40.2 mm was measured on 98/11/01 (Figure 3), but this occurred when aphid populations were already declining as a result of wheat ripening. Grain yields averaged 2170 (SD = 215.9) and 1976 (SD = 177.4) g/plot for treatments and controls, respectively, and did not differ significantly ($F = 1.919$ on 1,6 df; $P = 0.2153$).

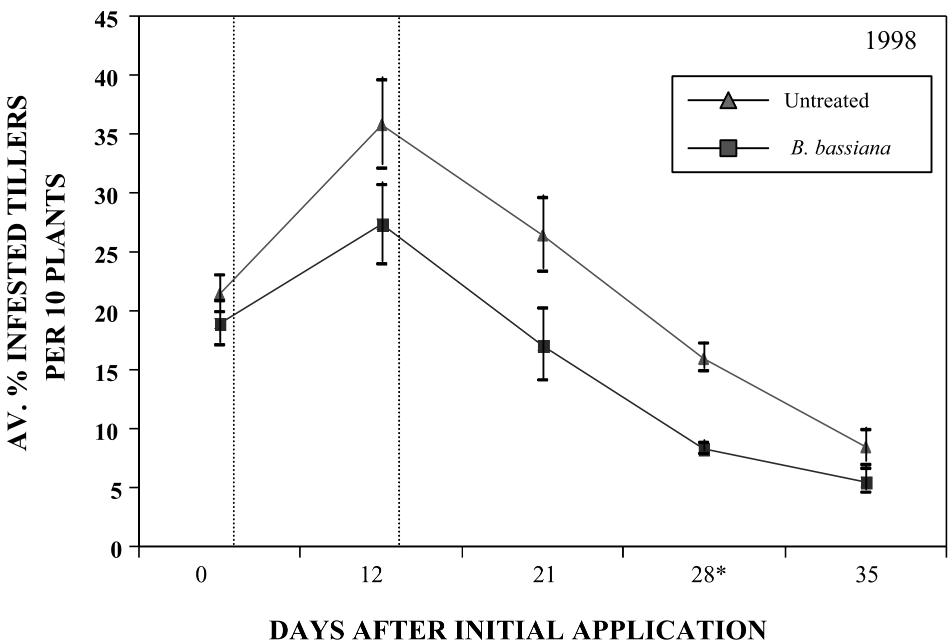

FIGURE 2. Mean percentage infested tillers recorded per 10 plants for trial in 1998. Bars represent SDM. Asterisks indicate significant difference between treatments and controls at the 5% level of significance. Dotted lines indicate Mycotrol® ES applications, i.e., on 98/10/02 and 98/10/15.

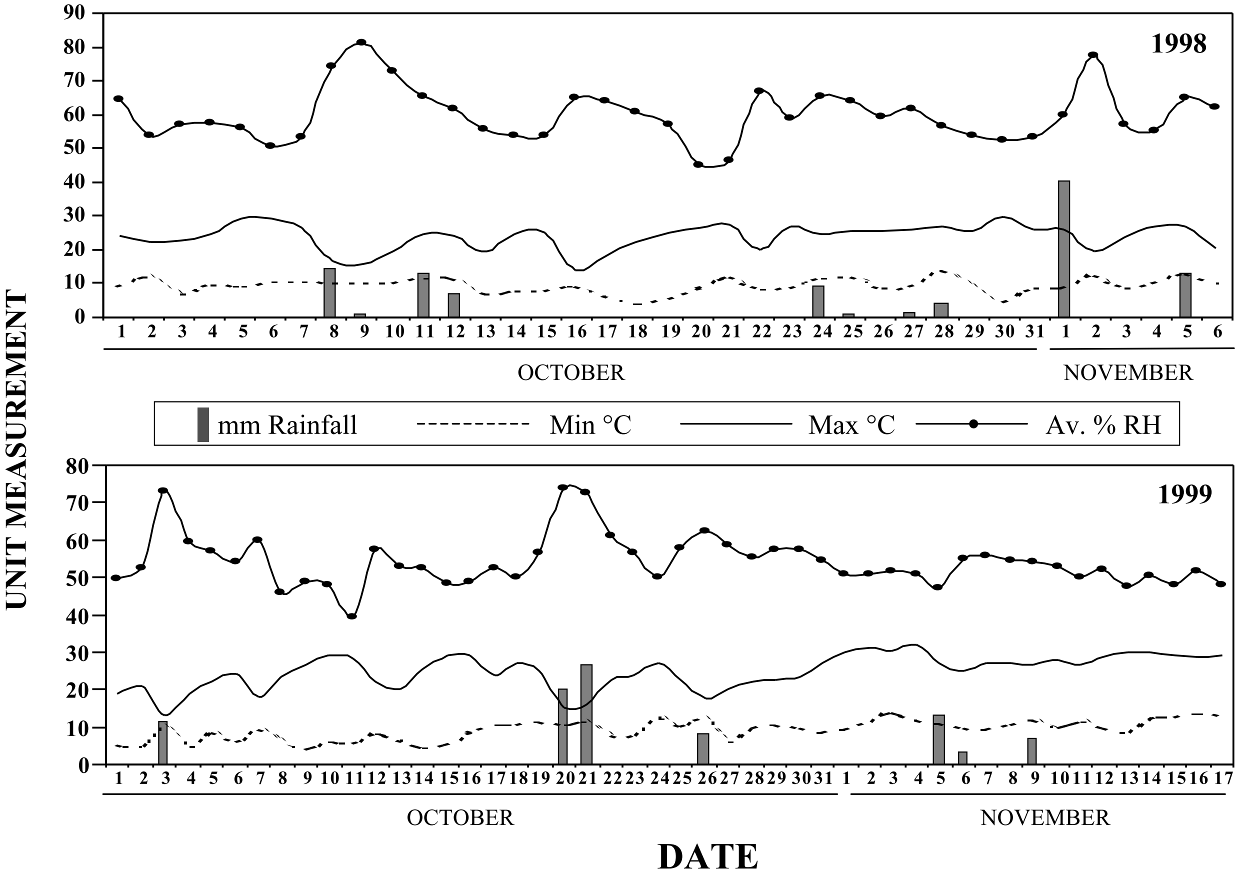

FIGURE 3. Daily weather data recorded for trials in 1998 and 1999.

1999 Trial

The number of aphids recorded on 99/10/04 (pre-spray), averaged 52.3 (SD = 31.3) with no differences among treatments and control ($P = 0.977$; Table 3). In contrast to the trend observed in 1998, the first Mycotrol® ES application on 99/10/05 (i.e., Treatment 1) resulted in no significant shift among aphid populations when compared with the (as then) unsprayed second treatment and/or control on 99/10/18 ($P = 0.809$; Table 3).

Compared with the control, the only significant difference in both the number of aphids ($P = 0.021$; Table 3) and percentage infested tillers ($P = 0.049$; Table 4) was observed 9 days after the single application on GS 39 (i.e., Treatment 2). However, in view of the rainfall events experienced during 1999 (resulting in very low aphid densities after 99/10/18) and the fact that no differences were detected between the two treatments (spray application on GS 31 & 39 versus GS 39 only) on any date post application (Tables 3 and 4), data for Treatments 1 and 2 were pooled (analysed as a single treatment) for each date.

The pooled data indicated a significant difference in the number of aphids recorded only on 99/10/29 ($P = 0.040$; Table 5); numbers in the treated plots were 63% lower than in the untreated plots. This level of population reduction (following a Mycotrol® ES application during the flag leaf stage) was attained despite a dramatic decline (> 80%) in aphid densities in all plots recorded between 99/10/18 and 99/10/29 (Figure 4). The only obvious explanation

TABLE 3. Mean number of *D. noxia* recorded per 10 plants pre- and post-application of Mycotrol® ES at a rate of 2.4 liters (5×10^{13} *B. bassiana* conidia) per hectare +0.1% Break-Thru® 1999

Date	99/10/04[a]	99/10/18	99/10/29	99/11/08	99/11/17
Control	52.5 ± 31.9a[b]	63.0 ± 47.3a	16.5 ± 1.0a	7.0 ± 3.6a	6.5 ± 3.1a
Two applications	51.3 ± 41.3a	55.3 ± 45.0a	9.3 ± 6.6ab	5.8 ± 5.6a	6.5 ± 2.5a
One application	53.3 ± 29.6a	65.5 ± 32.7a	3.3 ± 2.1b	3.5 ± 4.0a	3.8 ± 1.0a
P	0.977	0.809	0.021	0.295	0.189
F (2,9 df)	0.02	0.22	6.11	1.40	2.01

Treatments included 1) untreated control, 2) two fungal sprays applied 15 days apart on 99/10/05 and 99/10/20, and 3) one fungal spray applied 99/10/20.

[a]Pre-spray count.

[b]Log 10 transformation applied prior to ANOVA. Means ± standard deviations followed by the same letter within the same column are not significantly different by Tukey's HSD test at the 5% level.

TABLE 4. Percentage tiller infestation recorded per 10 plants pre- and post-application of Mycotrol® ES at a rate of 2.4 liters (5×10^{13} *B. bassiana* conidia) per hectare +0.1% Break-Thru® 1999

Date	99/10/04[a]	99/10/18	99/10/29	99/11/08	99/11/17
Control	27.6 ± 11.4a[b]	27.3 ± 11.8a	11.9 ± 6.8a	5.5 ± 1.1a	6.6 ± 1.5a
Two applications	25.3 ± 16.5a	22.7 ± 15.0a	8.3 ± 2.9ab	4.1 ± 2.5a	5.8 ± 4.0a
One application	26.8 ± 14.0a	31.7 ± 13.3a	3.6 ± 2.4b	3.0 ± 3.4a	3.6 ± 0.7a
P	0.957	0.631	0.049	0.253	0.167
F (2, 9 df)	0.04	0.48	4.30	1.61	2.20

Treatments included (1) untreated control, (2) two fungal sprays applied 15 days apart on 99/10/05 and 99/10/20, and (3) one fungal spray applied 99/10/20.

[a]Pre-spray count.

[b]Arcsine transformation applied prior to ANOVA. Means ± standard deviations followed by the same letter within the same column are not significantly different by Tukey's HSD test at the 5% level.

TABLE 5. Mean number of *D. noxia* recorded per 10 plants pre- and post-application of Mycotrol® ES at a rate of 2.4 liters (5×10^{13} *B. bassiana* conidia) per hectare +0.1% Break-Thru®. Pooled data (i.e., Treatment 1 and 2) for 1999

Date	99/10/04[a]	99/10/18	99/10/29	99/11/08	99/11/17
Control	52.5 ± 31.9a[b]	63.0 ± 47.3a	16.5 ± 1.0b	7.0 ± 3.6a	6.5 ± 3.1a
Treatments	52.3 ± 33.3a	60.4 ± 36.8a	6.3 ± 5.5a	4.6 ± 4.7a	5.1 ± 2.3a
Efficacy[c]	–	4.2	62.9	32.1	21.2
P	0.889	0.969	0.040	0.245	0.441
F (1,10 df)	0.02	0.002	5.60	1.52	0.64

[a]Pre-spray count. Spray applications administered on 99/10/05 and 99/10/20.
[b]Log 10 transformation applied prior to ANOVA. Means ± standard deviations followed by the same letter within the same column are not significantly different by ANOVA at the 5% level.
[c]Percent reduction in aphid population relative to the control.

for this phenomenon is the adverse weather conditions experienced on 99/10/20 and 99/10/21 (Figure 3). During this period, a total of 47 mm of rain was recorded, coinciding with an average temperature of only 13.3°C.

No significant difference in the percentage infested tillers was detected for the pooled data on any of the survey dates during 1999 (Table 6, Figure 5). However, at the 8% test level, a significant difference was observed on 99/10/29 (Table 6). Grain yields averaged 1846 (SD = 150.0), 1965 (SD = 78.2), and 1910 (SD = 135.7) g/plot for Treatments 1, 2 and controls, respectively, and did not differ significantly ($F = 0.913$ on 2,9 df; $P = 0.4353$).

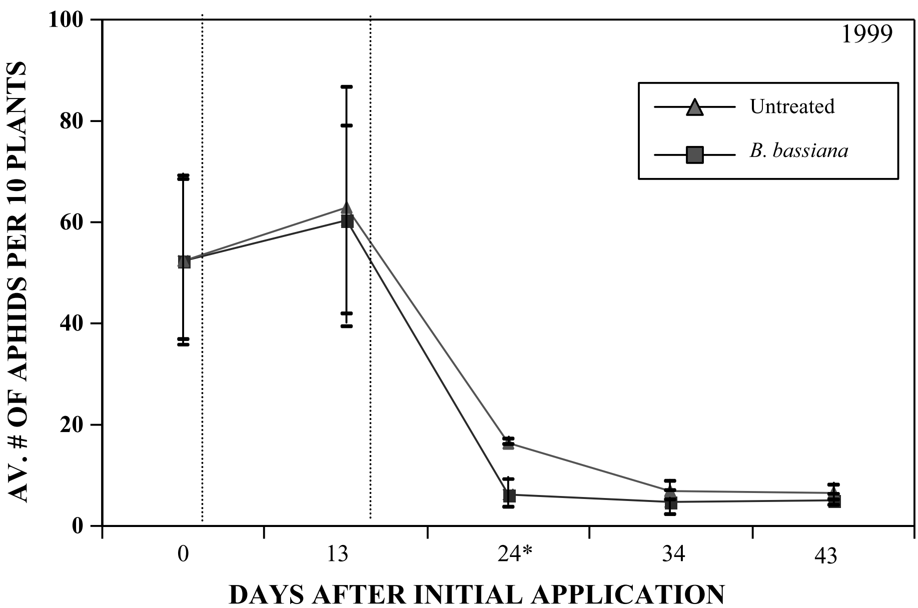

FIGURE 4. Mean number of aphids recorded per 10 plants for trial in 1999. Bars represent SDM. Asterisks indicate significant difference between treatments and controls at the 5% level of significance. Dotted lines indicate Mycotrol® ES applications, i.e., on 99/10/05 and 99/10/20.

TABLE 6. Percentage tiller infestation recorded per 10 plants pre- and post-application of Mycotrol® ES at a rate of 2.4 liters (5×10^{13} *B. bassiana* conidia) per hectare +0.1% Break-Thru®. Pooled data (i.e., Treatment 1 and 2) for 1999

Date	99/10/04[a]	99/10/18	99/10/29	99/11/08	99/11/17
Control	27.6 ± 11.4a[b]	27.3 ± 11.8a	11.9 ± 6.8a	5.5 ± 1.1a	6.6 ± 1.5a
Treatments	26.0 ± 14.2a	27.2 ± 14.0a	6.0 ± 3.5a	3.5 ± 2.9a	4.7 ± 2.7a
Efficacy[c]	–	0.4	49.6	36.4	28.8
P	0.800	0.963	0.080	0.211	0.159
F (1,10 df)	0.07	0.002	3.78	1.78	2.31

[a]Pre-spray count. Spray applications administered on 99/10/05 and 99/10/20.
[b]Arcsine transformation applied prior to ANOVA. Means ± standard deviations followed by the same letter within the same column are not significantly different by ANOVA at the 5% level.
[c]Percent reduction in tiller infestation relative to the control.

DISCUSSION

During 1997, a field trial was conducted at ARC-SGI using *B. bassiana* (Mycotrol® ES at 5×10^{13} conidia/ha + 0.03% Silwet surfactant) against the Russian wheat aphid feeding on susceptible wheat (cultivar 'Tugela') under dryland field conditions. The trial was designed to assess the impact of weekly applications of *B. bassiana* on aphid population growth compared with untreated controls. Following the first (on GS 31), second and third application, aphid densities counted 6 days later in treated plots averaged 22, 21 and 21 aphids per tiller, respectively. As expected, control plots showed an increase in aphid densities averaging 29, 38 and 51 aphids per tiller during the same survey dates. Therefore, the greatest reduction in the number of *D. noxia* (59% compared with the control) was observed following the third application, i.e., during the early flag-leaf stage (GS 38). Notably, an application after colonisation and subsequent rolling of the flag leaves (i.e., fourth

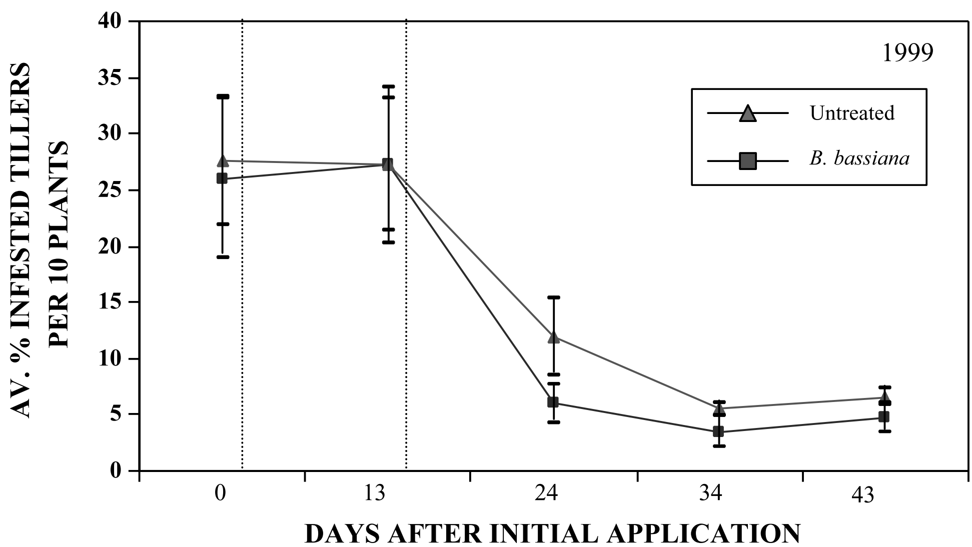

FIGURE 5. Mean percentage infested tillers recorded per 10 plants for trial in 1999. Bars represent SDM. Asterisks indicate significant difference between treatments and controls at the 5% level of significance. Dotted lines indicate Mycotrol® ES applications, i.e., on 99/10/05 and 99/10/20.

application on GS 45; boots swollen) did not prevent aphids from increasing significantly, from 21 to 45 aphids per tiller within 6 days (Hatting & Poprawski, 1998).

The most serious damage inflicted by *D. noxia* normally occurs during the period between the emergence of the flag leaf (GS 37) and the awn (GS 51) (Du Toit & Walters, 1984; Fouché *et al.,* 1984). For this reason, chemical control of *D. noxia* on susceptible wheat, is recommended at growth stage 31, when 4–7% plants are infested (Du Toit, 1986), thereby protecting the upper two leaves from aphid infestation (Kriel *et al.,* 1984). Although the first mycoinsecticide application was correctly timed in terms of this threshold (applied at GS 31), protection of the upper leaves was not satisfactory even though subsequent applications were made at weekly intervals. It was concluded that the poor level of control throughout the critical flag-leaf stage was mainly attributed to the rapid increase in aphid numbers after colonization of the flag leaf (Kriel *et al.,* 1986) and aphid habitation of a cryptic site (within the rolled flag leaves) which possibly shielded the aphids from subsequent spray applications.

It was hypothesized in the present study that the use of a mycoinsecticide in combination with host plant resistance would largely address the above-mentioned impediments. Over the duration of the 1998 trial, ca. 65% (corrected) fewer aphids were observed in treated plots compared with controls. A similar level of population reduction was observed during the 1999 trial; however, treatment effects were only briefly evident due to a rapid field-wide decline in aphid populations caused by adverse (cool, wet) weather conditions. Surprisingly, however, Vandenberg *et al*. (2001) observed no significant efficacy of *B. bassiana* strain GHA applied against *D. noxia* on the resistant wheat cultivar 'IDO-488'. Vandenberg *et al.* (2001) suggested that the reduced leaf-rolling response on the resistant variety might have resulted in a microclimate less favourable to fungal infection of *D. noxia*. However, observations of similar levels of control on both susceptible (Hatting & Poprawski, 1998; Vandenberg *et al.,* 2001) and resistant varieties (present study) suggest either that plant architecture is not an important factor determining fungal efficacy, or that greater exposure of aphids to spray applications and the loss of favourable (shaded, humid) conditions due to reduced leaf roll have offsetting effects. Perhaps more importantly, the actual timing of fungus application needs to be considered. As pointed out above, a late fungus application after colonization and subsequent rolling of the flag leaves (GS 45) did not prevent aphids from increasing significantly on the susceptible cultivar (Hatting & Poprawski, 1998). Unfortunately, no indication of the actual timing of application in terms of wheat growth stage was given by Vandenberg *et al*. (2001). Most effective control during the ARC-SGI 1997 trial was observed following an application during the early flag leaf stage (GS 38) when little leaf roll was evident and aphids were actively migrating onto the flags. This was the case also in the 1999 trial reported here. The treatment including both an early (GS 31) and late (GS 39) application had no greater effect than the treatment with a late application only (the early treatment had no apparent effect).

These observations and observation of aphid infestation dynamics lead to an alternative hypothesis that might account for greater efficacy of fungal sprays applied during colonization of the flag leaf. Aphids migrating to the flag leaf may acquire lethal doses of conidia from the plant surface (Hall, 1979; Roditakis *et al.,* 2000). The greater change in aphid numbers that occurred following the initial spray in 1998 (significant at the 8% level; $P = 0.075$; corrected efficacy of 56%; Table 1) than in 1999 ($P = 0.97$; efficacy of 4%; Table 5) may also support this hypothesis, as the cultivar used in 1998 ('Limpopo') supported a higher level of aphid activity than did the cultivar used in 1999 ('Elands'). On cultivar 'Limpopo', the average percentage tiller infestation in untreated plots increased from 21% (pre-spray) to 36% within 13 days (Table 2). In contrast, infestation of tillers of cultivar 'Elands' remained virtually unchanged during the comparable period of time in 1999 (Table 6). During these two time periods, ambient temperature conditions were highly comparable, with daily average minimum and maximum temperatures recorded for 1998 and 1999 ranging from 9.7 to 23°C and 7.1 to 24.5°C, respectively. Although four rainfall events were recorded during

1998, these were of low intensity, and averaged only 8.9 ± 6.1 mm per event. No rainfall was recorded during this time period for 1999. The higher level of migration observed on cultivar 'Limpopo' is more likely attributed to the lower level of antibiotic resistance in that cultivar compared with cultivar 'Elands', as recently demonstrated by Basky (2002). Aphid numbers in untreated plots increased 3.5-fold in 13 days on cultivar 'Limpopo' compared with a 1.2-fold increase over a 14 day period on cultivar 'Elands' (Tables 1 and 5).

The above discussion provides a possible explanation as to why the 1999 treatment with two applications of *B. bassiana* was no more efficacious than the treatment with a single application (why the early application in the 1999 trial was apparently ineffective). At the time of the early spray (GS 31), most of the aphids on the 'Elands' cultivar may have been immobile and residing in cryptic sites protected from the spray application. In view of the many different resistant cultivars commercially available in South Africa (Tolmay, 2002), studies aimed at better understanding the tritrophic interactions between host plant, pest and pathogen are warranted.

Data gathered during the 1998 season with cultivar 'Limpopo' showed an average yield of 2.11 and 1.97 tons/ha for sprayed (one foliar application of metasystox + parathion; see Nel *et al.,* 1999) and untreated plots, respectively (ARC-SGI, unpublished data). These figures compare favourably with the ones obtained with the 1998 mycoinsecticide trial reported here, i.e., 2.17 and 1.98 tons/ha for fungus-treated and control plots, respectively. Moreover, although this difference in yield (fungus-treated versus control) was not statistically significant ($P = 0.2153$), the slightly higher yield obtained with the fungus-treated plots amounted to *ca*. ZAR150/ha given an average wheat market value of ZAR810/ton during that season. By using mean-separation analyses, Nault and Kennedy (1998) also found a weak relationship between crop yield and defoliation by *Leptinotarsa decemlineata* (Say) on potatoes, suggesting that these procedures do not have sufficient statistical power to detect small reductions in yield.

Only two other reports on field trials with a hyphomycete against Russian wheat aphid have been published. Knudsen and Wang (1998) used pellet-formulated *B. bassiana* (Knudsen *et al.,* 1990) in four trials over two seasons. Although $>95\%$ of pellets were observed to have sporulated on the soil surface during one trial, a maximum high of only 18% mortality was recorded. The restricted movement of infective propagules into the crop canopy was considered the main reason for this low level of control obtained. The previously mentioned trial by Vandenberg *et al*. (2001) included two foliar-applied formulations of *B. bassiana* and *Paecilomyces fumosoroseus* (Wize) Brown & Smith under irrigated conditions against *D. noxia* feeding on susceptible wheat. They observed reduction in aphid population densities within 14 days post application in most experiments with spring wheat for three consecutive years (1995–1997). The highest level of control recorded by these authors during their 1997 trial (i.e., large-plot experiment; single application) was ca. 60% 14 days post application, comparable with the 56% (corrected; 1998 trial) reported here.

The rainfall events experienced during 1999 resulted in very low aphid densities after 99/10/18. Densities averaged only 0.77 aphids per plant during the following three survey dates, most probably dissembling treatment effects. Similar 'knock-down' effects due to rainfall have been reported for *D. noxia* feeding on susceptible wheat (Kriel *et al.,* 1984, 1986). However, on susceptible wheat, aphid populations often showed a dramatic increase after such events (Kriel *et al.,* 1984, 1986), a phenomenon ascribed, in part, to the improvement in nutrient status of the host plant (Kriel *et al.,* 1986). A similar aphid resurgence was not observed on the antibiotic host plant, suggesting that natural weather events may play an important role in regulating *D. noxia* populations on such cultivars. In fact, the open leaf architecture of resistant cultivars may augment the physical impact of rainfall on resident aphid populations.

Observations from this study suggest that aphid activity, which directly exposes the aphids to spray applications and/or leads to secondary pick-up of fungal inoculum, may play an important role in the timing of mycoinsectide applications against *D. noxia*. In this regard, migration of *D. noxia* onto the flag leaves should be further investigated as a behavioural trait for possible exploitation when considering the use of a mycoinsecticide. The concept of increased insect movement leading to enhanced secondary pick-up of fungal inoculum is supported by various researchers (Griffiths & Pickett, 1980; Furlong & Pell, 1996; Shah *et al.,* 1999; Roditakis *et al.,* 2000). However, as noted during these trials, genotypic variation among cultivars will most likely affect the level of aphid activity, and hence, the level of control following an early application (GS 31) of the fungus. On the other hand, an application of the fungus during the early flag-leaf stage (GS 39) may result in a more reliable level of control, as was observed during 1998 (75% efficacy) and 1999 (63% efficacy) 8–9 days post application, using different aphid-resistant cultivars.

Oils and organosilicone surfactants are well known to possess insecticidal activity, especially against soft-bodied insects such as aphids (Davidson *et al.,* 1991; Poprawski *et al.,* 1999). Unfortunately, potential effects of the spray carrier adjuvants were not examined in these studies and, to our knowledge, this also has not been investigated by any other researchers who have tested fungal pathogens against cereal aphids. In a recent greenhouse study at ARC-SGI, applications of the spray carriers used in these trials against *D. noxia* on potted wheat held under open-air conditions produced ca. 16% mortality (Hatting, 2002). Future studies should include spray-carrier controls to quantify the impacts of these materials under actual field conditions.

The levels of control achieved during these trials (60–65%) are clearly inadequate to recommend use of *B. bassiana* as a stand-alone aphid control agent. The results do, however, demonstrate potential for use of this pathogen as one component of a multi-component integrated-control programme including resistant wheat varieties. Issues in need of further investigation include economic threshold values for individual resistant cultivars, large-scale field application (tractor and/or aerial), and development of local mycoinsecticide products with high efficacy towards not only *D. noxia*, but also the other five cereal-aphid species attacking wheat in South Africa.

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